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# Correlation among phenyltins molecular properties, degradation and cellular influences on *Bacillus thuringiensis* in the presence of biosurfactant

Litao Tang<sup>a</sup>, Linlin Wang<sup>a</sup>, Huase Ou<sup>a</sup>, Qusheng Li<sup>a</sup>, Jinshao Ye<sup>a,\*</sup>, Hua Yin<sup>b,\*</sup><sup>a</sup> Key Laboratory of Environmental Exposure and Health of Guangzhou City, School of Environment, Jinan University, Guangzhou 510632, China<sup>b</sup> College of Environment and Energy, South China University of Technology, Guangzhou 510006, China

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## ABSTRACT

Although a successive dearylation is recognized as a triphenyltin biodegradation pathway, the cleavage pattern of the various chemical bonds of phenyltins is still not clear. Moreover, the correlation among phenyltins biosorption, degradation, molecular properties and metabolic impacts is far from fully understood. Therefore, phenyltins treatment of *Bacillus thuringiensis* was conducted. After degradation for 7 d in the presence of 50 mg L<sup>-1</sup> of the surfactant sucrose fatty acid ester, the degradation efficiency of 1 mg L<sup>-1</sup> triphenyltin reached its peak value of 89%. The surfactant altered the topological structure of the cellular peptide chains, accelerated triphenyltin binding and transport, increased cellular viability, Na<sup>+</sup>/K<sup>+</sup>- and Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase activities, increased PO<sub>4</sub><sup>3-</sup> and Na<sup>+</sup> assimilation, and decreased K<sup>+</sup> and Mg<sup>2+</sup> release, resulting in the enhancement of triphenyltin degradation. However, surfactant did not change the successive dephenylation pathway, which was primarily determined by the bond energy of each Sn–C bond of triphenyltin

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## 1. Introduction

Triphenyltin (TPT), an endocrine disruptor, is highly toxic to various invertebrates and vertebrates [1], and it is polluting the global environment as it is used worldwide. The high hydrophobicity and low solubility of TPT limit its access to microbes, resulting in its persistence in the ecosystem with a half-life varying from weeks to years [2]. Surfactants with hydrophobic and hydrophilic domains are capable of lowering TPT surface tension and enhancing its solubility, consequently leading to its high bioavailability. For example, some surfactants, such as sodium dihexylsulfosuccinate [3], rhamnolipid [4] and tea saponin [5], are found to obviously enhance organotin biodegradation. However, information regarding the influence of these surfactants on TPT molecular properties, biodegradation pathways, metabolite transformation, cell wall alteration and cellular metabolic responses is still very limited.

As an enzyme inhibitor and metabolic disproportionation compound [6], TPT may depress the metabolic activities involving its transport and degradation. Therefore, it is vital to investigate the

regulation of cellular affinity, metabolism, and membrane permeability in the presence of surfactants during the TPT biodegradation process. As a biosurfactant with high biodegradability, surface-active properties and a wide use in the food, pharmaceutical and cosmetics industries [7], sucrose fatty acid ester (SFAE) was selected as a representative surfactant in the current study.

TPT biodegradation has been attributed to the joint processes of biosorption, transport and transformation. Among these steps, biosorption is an initial reaction and may be the limited step of TPT degradation because the structural and physicochemical relations between phenyltins (PTs) and the effective groups in the bacterial peptidoglycan layers play a key role in PTs binding, uptake and transformation [8]. Excessive alteration of the peptidoglycan structure induced by PTs could trigger significant apoptosis, whereas a lack of affinity between the cellular surface and PTs would limit PTs binding and transport. Thereby, it is necessary to investigate TPT binding to the cell surface.

To date, only a small number of bacterial strains have been identified as PTs-degrading microorganisms, such as *Bacillus thuringiensis* [5], *Brevibacillus brevis* [4] and *Stenotrophomonas maltophilia* [9]. However, the previous reports do not reveal why TPT transformation to monophenyltin (MPT) by these strains was faster than the further degradation of the produced MPT. The impact of different concentrations of surfactant on cellular metabolism,

\* Corresponding authors.

E-mail addresses: [jsye@jnu.edu.cn](mailto:jsye@jnu.edu.cn) (J. Ye), [huayin@scut.edu.cn](mailto:huayin@scut.edu.cn) (H. Yin).

cell wall structure, TPT transport, biosorption and transformation has seldom been illustrated. Therefore, the present work investigated TPT biosorption mechanisms and degradation pathways in the presence of SFAE and the correlation between degradation and molecular properties of pollutants. The viable biomass, ATPase activities, ion metabolism and cell morphology were analyzed to reveal cellular metabolic responses during the TPT degradation process by *B. thuringiensis*. The affinity between the cell wall peptidoglycan and TPT enhanced by SFAE was computed using ChemBio3D Ultra 13.0.

## 2. Materials and methods

### 2.1. Strain and chemicals

*B. thuringiensis* was isolated from organotin-bearing sediments collected at an e-waste processing town, Guiyu, in Guangdong Province, China. Triphenyltin chloride (purity=98.8%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The concentrations of beef extract, peptone, NaCl and MgSO<sub>4</sub> in the culture medium were 3, 10, 5 and 0.03 g L<sup>-1</sup>, respectively. The mineral salt medium (MSM) for TPT degradation contained (in mg L<sup>-1</sup>) 150 Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 50 KH<sub>2</sub>PO<sub>4</sub>, 30 NH<sub>4</sub>Cl, 5 Zn(NO<sub>3</sub>)<sub>2</sub> and 5 MgSO<sub>4</sub>.

### 2.2. Microbial culture

*B. thuringiensis* was inoculated into 250 mL Erlenmeyer flasks with 100 mL culture medium at 30 °C on a rotary shaker at 130 rpm for 12 h. Subsequently, the cells were separated from the medium by centrifugation at 3500 × g for 10 min and were then washed three times with distilled water sterilized at 121 °C for 30 min.

### 2.3. TPT solubility enhancement by SFAE

The enhanced solubility of 1 mg L<sup>-1</sup> TPT by SFAE at different concentrations up to 1000 mg L<sup>-1</sup> was performed at 25 °C in 20 mL MSM by shaking on a rotary shaker at 100 rpm for 24 h. After equilibration for 12 h in the dark, the MSM was centrifuged at 3500 × g for 10 min. Subsequently, 10 mL of supernatant was transferred and extracted twice by hexane in an ultrasonic bath. The soluble TPT in the supernatant was detected according to Section 2.5.

### 2.4. TPT biodegradation, biosorption and adsorption

Twenty microliters of MSM containing 1 mg L<sup>-1</sup> of TPT, the initial dosage of *B. thuringiensis* at 0.3 g L<sup>-1</sup> and different concentration of SFAE (0–80 mg L<sup>-1</sup>) were inoculated into a 250 mL Erlenmeyer flask in the dark at 25 °C on a rotary shaker at 100 rpm for 7 d. After degradation, the cells were separated and washed using phosphate buffer solution (PBS) for 20 min. TPT in PBS and inside of the cells represents TPT biosorption and accumulation, respectively. The total residual TPT in the MSM and cells was used to determine the degradation efficiency. To determine passive TPT binding by the cell surface, adsorption by dead cells inactivated by 2.5% glutaraldehyde for 24 h was evaluated under the same conditions. The controls were run in parallel in flasks that were not inoculated. All of the experiments were performed in triplicate, and the mean values were used in the calculations.

### 2.5. Analytical methods of PTs

After TPT degradation, PTs were analyzed by gas chromatography–mass spectrometry (QP2010, Shimadzu) equipped with a type Rxi-5MS GC column (30 m × 0.25 mm × 0.25 μm) [4]. Briefly, a constant flow rate of the carrier gas helium was set at 1.1 mL min<sup>-1</sup>. The column

temperature program started at 50 °C and held for 1.5 min. Subsequently, the oven was heated to 300 °C at a rate of 10 °C min<sup>-1</sup> and held for 4 min. The solvent cut time was set to 2.6 min. The GC–MS interface temperature was maintained at 280 °C. Mass spectra were recorded at 1 scan s<sup>-1</sup> under electronic impact with an electron energy of 70 eV and a mass range from 50 to 650 atoms to mass unit. The temperature of the ion source was set at 250 °C. Samples of 2 μL were injected directly.

### 2.6. Cellular morphology

The morphology of the cells before and after TPT degradation was observed by atomic force microscopy (AFM) and scanning electronic microscope (SEM) (Philips XL–30E). Briefly, the cells were deposited onto a glass surface and then air-fixed. Subsequently, the samples were mounted onto the XY stage of a commercial Auto-probe CP AFM, and the integral video camera was used to locate the observed regions. The imaging experiments were conducted in the tapping mode using a microfabricated silicon cantilever (Park Scientific Instruments).

Before SEM observation, the samples were fixed by glutaraldehyde for 24 h and sequentially dehydrated by ethanol at 20%, 50%, 70%, 90% and 100% (v/v). Then, the samples were immersed in isoamyl acetate for 30 min before dryness at the breakthrough point using CO<sub>2</sub> as a refrigerant. Subsequently, the samples were coated with gold to strengthen the conductivity and photographed by SEM.

### 2.7. Ion assimilation and release during TPT degradation

After degradation for 7 d, the cells were centrifuged at 3500 × g for 10 min. The resultant supernatant was filtered using a 0.22 μm polyether sulfone filter, and the concentrations of PO<sub>4</sub><sup>3-</sup>, Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup> were detected using an ICS–900 ion chromatography system (Dionex, Sunnyvale, USA). The change in the ion concentration in MSM was calculated as the experimental content minus the control level; therefore, a negative value represents ion assimilation and a positive one represents ion release by the cells.

### 2.8. Analytical methods for ATPase activities

After TPT degradation, the cells were washed three times in cold PBS (pH 7.4), suspended in a cold lysis buffer (30 mM Tris–HCl, 7 M urea, 2 M thiourea, and 4% w/v 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate at pH 8.5) and lysed by sonication in an ice bath for 15 min. The cellular debris was removed from the suspension at 16,000 g for 5 min at 4 °C. Na<sup>+</sup>/K<sup>+</sup>- and Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase activities were analyzed according to the instructions of the test kit, which was provided by the Nanjing Jiancheng Bioengineering Institute, China.

### 2.9. Cellular activities in carbon nutrient use after TPT degradation

Biolog microplates were used to analyze the cellular activities in carbon nutrient use after TPT degradation. The plates contained 96 wells with different carbon nutrients and a blank well without substrate. Each well had the redox dye tetrazolium, which could be reduced by the NADH generated by cellular metabolism. Briefly, 1 mL MSM was mixed with 99 mL of 0.85% sterilized saline solution. Next, 150 μL of the solution was inoculated into each well of the Biolog microplate and incubated at 25 °C in the dark. The optical density at 590 nm of each well was determined every 12 h. The Biolog data were calculated using an average well color development (AWCD) method.

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